Comm.
Dr. Andervont
Dr. Gardner
Dr. Huebner

CARCINOGENESIS

#920

JUL 13 1973

1. Principal Investigator (give title and degrees)

William F. Benedict, M.D.
Assistant Professor of Pediatrics,
USC School of Medicine

2. Institution and Address:

Divisions of Hematology-Oncology and Medical Genetics,
Childrens Hospital of Los Angeles
4650 Sunset Boulevard,
Los Angeles, California. 90054.

3. Department(s) where research will be done or collaboration provided

Divisions of Hematology-Oncology and Medical Genetics,
Childrens Hospital of Los Angeles

4. Short title of study:

a. Relationship of chromosomal changes to human malignancy in a human-mouse/human-hamster model system.

b. Chromosome changes, metaplasia and carcinogenesis in hamster tracheal organ culture.

5. Proposed starting Date:

December 1, 1973

6. Estimated time to complete:

Two years.

7. Brief description of specific research aims:

A. Firstly we shall attempt to study the importance of specific human chromosomes in malignancy by the use of a human-mouse or human-hamster model system recently developed. Both near diploid human carcinomas and sarcomas will be injected into mice or hamsters which have been previously treated with anti-thymocyte serum. Chromosomal analysis will be made of the tumor cells to

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identify similar increases or decreases in particular chromosomes using a trypsin-Giemsa banding technique which enables each human chromsome to be easily identified (10). Revertant cells will be isolated which have decreased tumor producing potential and their chromosomal pattern will also be studied. Finally, all tumor lines established at the Childrens Hospital of Los Angeles over the next two years will be similarly studied in the human-hamster and/or human-mouse system.

B. Secondly, we shall study hamster tracheal epithelial tissue in organ culture. Mary Baker from Dr. Michael Sporn's laboratory at the Lung Cancer Unit of the NCI will be joining our group in September. She has been one of the pioneers in developing this system and now hamster tracheal tissue can be maintained for extended periods.

We shall use this system to study the evolution of epithelial metaplasia and hopefully carcinoma <u>in situ</u> by testing these cultures with various carcinogens, particularly polycyclic hydrocarbons: Chromosomal analysis will be done on the early changes toward malignancy to see if the same "expressor" and "suppressor" chromosomes are involved as has previously been reported in chemically induced transformation of hamster embryo cultures (3). These studies are a matural retention of other CTR projects being done in collaboration with Dr. R. Kouri and Dr. C. Whitmine at Microbiological Associates on endotracheal produced polycyclic hydrocarbon carcinogenesis in the mouse.

8. <u>Background</u>

A. The importance of the balance of specific hamster "expressor" and "suppressor" chromosomes in virally (1) or dimethylnitrosamine (2) produced malignant transofmration has recently been reported. We have found the same "expressor" and "suppressor" chromosome involvement in 1-β-D-arabinofuranosylcytosine (ara-C) transformation as previously had been found with dimethylnitrosamine (3). Thus it will be interesting to see whether or not a similar chromosomal relationship will be found with other chemical carcinogens such as polycyclic hydrocarbons. We have also recently begun studying chromosomal changes in methylcholantrene transformed C_3H mouse cells. Preliminary data suggests the presence of "suppressor" chromosomes in the mouse system as well. Threfore, since there is evidence of chromosomes which may influence the expression of malignancy in two mammalian systems, it is important to see if a

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similar relationship exists in human tumors.

A model system has recently been described which should expediate this investigation (4). Human malignant cells in tissue culture have been able to produce tumors readily in NIH Swiss mice treated with antithymocyte serum. Epithelial cells derived from human carcinomas produce carcinomas in the mouse, whereas fibroblastic cells from sarcomas yield sarcomas (Paul Arnstein, personal communication). A lung carcinoma and a sarcoma both of which are near diploid have been made available for our initial studies. They produce rapidly growing carcinomas and sarcomas respectively in the mouse (Paul Arnstein, personal communication). We have already used chromosomal changes as criteria for the diagnosis of human malignancy in solid tumors and effusions (5-7). If specific chromosomal changes could be recognized, this would facilitate chromosomal diagnosis of malignancy in humans as well as increase our understanding of etiological factors in human cancer.

Since growing human tumor cells in the mouse has yielded C-type RNA particles in the human cells, whereas growing the cells in newborn hamsters has not thus far (Robert McAllister, personal communication), we shall grow the tumor cells in both of these systems.

B. It is only recently that hamster or rat tracheal tissue has been grown with success in long and short term organ culture without changing morphological and biochemical functions (8,9). It has been shown that H-benzo(a)pyrene binds to DNA more extensively than controls in the presence of F_2O_3 and less in the presence of 7,8-benzoflarone than in controls (8). Also, early metaplastic-like morphological changes have been seen in longer term rat tracheal cultures following exposure to polycyclic hydrocarbons (9). Thus, a similarity to the in vivo situation has been established. We hope to use this similarity to study the relationship of early chromosome changes in the system to the in vivo development of malignancy.

9. Experimental Design:

A. Initially a near diploid long carcinoma cell line and a sarcoma cell line will be studied. Chromosomal analysis will be done before and after producing tumors using the trypsin-Giemsa

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banding technique (10). Human tumors will be established in mice and hamsters by treating the animals with antithymocyte or antilymphocyte serum (4). Pathological diagnosis will be made on the tumors and expression of RNA-virus monitored by the reverse transcriptase assay. Tumors will be placed back in cell culutre. Clones of cells will then be selected which have lower tumor producing potential and these clones will be evaluated for change in chromosome complement from the tumor producing clones . Subsequently all tumor lines isolated at the Childrens Hospital will be evaluated in a similar fashion.

B. Hamster tracheal tissue in organ culture will be exposed to both high dose short term and low dose long term treatment of polycyclic hydrocarbons and ara-C. Simultaneous histological and chromosome studies will be done at various times after exposure, as has been described in part A.

10. Space and Facilities available:

Complete laboratory for karyotyping
Appropriate laboratory equipment
Dark room for developing and printing film
Adequate animal facilities

11. Additional Facilities Required:

None

12. Biographical sketches of investigator(s) and other professional personnel:

(Appended)

13. Publications:

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First Year Budget:

A. Salaries:

NAME	% TIME	AMOUNT
William F. Benedict, M.D.	20	-0-
Peter Jones, Ph.D.	20	-0-
Hart Isaacs, M.D.	5	-0-
Benjamin Landing, M.D.	5	-0-
Mary Baker	80	10,000
Betty Paul	20	3,000
Corey Mark	20	1,500
	Sub-total for A.	14,500

B. Consumable Supplies:

Antithymocyte and Antilymphocyte serum (3.75/test animal), 2,200
Animals (hamsters, and mice), 1,200
Tissue Culture Dishes, 1,000
Medium and serum, 800
Film and Processing, 400

Sub-total for B. 5,600

C. Other Expenses:

Travel (To Microbiological Associates, Bethesda, Maryland for Principal Investigator).

Sub-total for C. 600 20700.

D. Permanent Equipment:

Laminar Flow Hood, 650
CO_2 Incubator, 1,200

Sub-total for D. 1,850

E. Indirect Costs:

37.62% of salaries and wages per DHEW Agreement dated February 23, 1972. 5,050 3105

TOTAL REQUEST: 27,600 25655.

Estimated Future Requirements:

Year 2	Salaries	Supplies	Other	Equipment	Ind. Cost	Total
	$15,400	$6,000	$600	-0-	$5,353	$27,353

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2. Yamamoto, T., Rabinowitz, Z., and Sachs, L.: Identification of the Chromosomes that control malignancy. Nature (in press)

3. Benedict, W.F., Kouri, R.: The relationship between 1-β-D-arabinofuranosylcytosine (ara-C) transformation and chromosomal changes in hamster fetal cells. 13th International Genetic Congress, Immunogenetics and Oncogenetics, 1973.

4. Todaro, G.J., Arnstein, P., Parks, W.P., Lennette, E.H., and Huebner, R.J.: A type-c virus in human rhabdomyosarcoma cells after innoculation into NIH Swiss mice treated with antithymocyte serum. Proc. Nat. Acad. Sci. USA 70:859-862, 1973.

5. Benedict, W.F., Porter, I.H., Brown, C.D., et al: Cytogenetic diagnosis of malignancy in recurrent meningioma. Lancet 1:971-973, 1970.

6. Benedict, W.F., Brown, C.D., Porter, I.H.: Long acrocentric marker chromosomes in malignant effusions and solid tumors. N.Y. State J Med 71:952-955, 1971.

7. Benedict, W.F., Porter, I.H.: The cytogenetic diagnosis of malignancy in effusions. Acta Cytol. 16:304-306, 1972.

8. Genta, V.G., Kaufman, D.G., Sporn, M.B. and Saffiotti, U.: Binding of ^{3}H-benzo(a)pyrene to DNA from hamster tracheal epithelial cells. Proc. Amer. Association for Cancer Res. 14:57, 1973.

9. Lane, B.P. and Miller, S.L.: Benzo(a)pyrene-induced changes in tracheal epithelium in organ culture. Fed. Proc. Abs. 3423, 1973.

10. Seabright, M.: A rapid banding technique for human chromosomes. Lancet II:971-972, 1971.

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Other Sources of Financial Support:

CURRENTLY ACTIVE

TITLE OF PROJECT	SOURCE & NUMBER	AMOUNT	INCLUSIVE DATES
Malignant Transformation of DNA Inhibitors	NCI-CA14226-01	$120,700	2-1-73 - 1-31-76
Cytogenetic Diagnosis and Etiology of Human Cancer	NCI-CA15039-01	$75,000	6-30-73 - 8-31-76
Chromosome Changes in Malignancy in the Mouse	Council for Tobacco Research	$10,000	7-1-73 - 6-30-74

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